Utilizing hydrogen carrier gas for sensitive analysis of pesticides in food using gas chromatography mass spectrometry

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Abstract

Purpose: To demonstrate the performance of the Thermo Scientifc™ HeSaver-H2Safer™ kit for iConnect™ split-splitless (SSL) injection module for trace analysis of pesticides using H_2 as a carrier gas as a safe and sustainable solution for laboratory operations

Methods: In this study, hydrogen was used as a carrier gas for GC-MS/MS analysis of trace levels of pesticides in food. The GC was fitted with a new hydrogen safer split/splitless injector, with chromatographic separation performed on a TG-5 SIL MS(30 m × 0.25 mm, 0.25 µm) capillary GC column. The chromatographic conditions applied ensured critical pair separation was achieved. The GC-MS/MS system was equipped with an advanced electron ionization (AEI) source to increase sensitivity for analyte detection. Quantification and assessment of critical regulatory requirements, such as linearity and sensitivity, were performed in a single software.

Introduction

Laboratories are under constant pressure to deliver results that are compliant with regulations. Helium (He) is an ideal carrier gas in GC-MS operations due to its inert nature and fast pumping efficiency. However, the dwindling global gas supply of He has created challenges for laboratories to maintain operations from economic and productivity standpoints. To mitigate these challenges, laboratories have looked to switch their carrier gas to an a more abundant alternative such as Hydrogen (H_2). Although additional challenges arise in using H_2 such as:

- Safety hazard posed by high flammability of H_2 - sensors required in GC oven for gas supply / heating shutdown when leaks are detected
- Reactivity of H_2 in the hot SSL inlet might lead to analyte degradation
- Decreased sensitivity and higher LODs due to increased noise from lower pumping efficiency of H_2 – reducing analytical performance towards reaching regulatory requirements
- Re-optimization bd validation of methods when switching carrier gas from He to H_2

Despite these challenges, laboratories are still faced with gas supply uncertainty and rising costs. Thus, technical solutions are needed to mitigate these challenges without compromising instrument performance and data quality.

Materials and methods

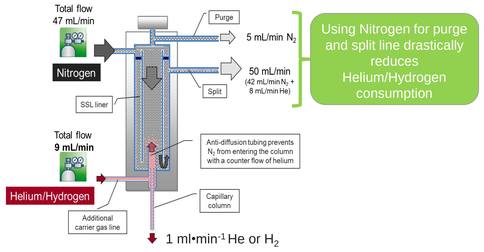
Standard and Sample Preparation

A 10-gram sample was extracted using the QuEChers method[1] In brief, acetonitrile was added to the sample material followed by the addition of magnesium sulphate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate (Thermo Scientific™ QuEChERS EN 15662 Method Extraction Kit). After mixing and centrifugation, a 5-mL aliquot of supernatant was mixed with magnesium sulfate and PSA sorbent, centrifuged again, and transferred to an amber GC vial and acidified with formic acid prior to analysis. A matrix matched calibration curve was prepared for both matrices in a concentration range of 0.005 – 0.500 mg•kg^{-1}.

Instrument configuration

Sample analysis was performed on the Thermo Scientific™ TSQ™ 9610 mass spectrometer with the same GC and injector configuration as described for the PAH analysis. Chromatographic separation was preformed on a Thermo Scientific™ TG-5SilMS capillary GC column (20 m × 0.18 mm, 0.18 µm) using hydrogen as a carrier gas at a flow of 1.2 mL•min^{-1} The TSQ 9610 mass spectrometer was operated in timed-SRM mode with all transitions optimized with Thermo Scientific™ AutoSRM software to obtain the highest possible sensitivity. Optimized ion transitions for H_2 along with injector, oven and mass analyzer conditions have been previously described[1]. Figure 1 shows the Schematic overview of HeSaver-H2Safer SSL injection module.

Figure 1. Schematic overview of HeSaver-H2Safer SSL injection module

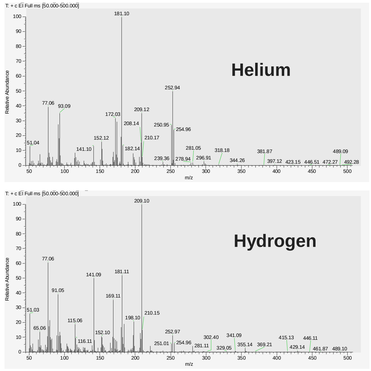


Results

Consequences of switching from Helium to Hydrogen

Using hydrogen instead of helium for chromatographic separation can shifts retention times. Despite resulting in generally shorter runtimes, the varying retention shifts for different analytes (in this study retention time shifts between 0.5 and 2 minutes were observed depending on the overall elution time of a given compound), requires initial confirmation of new retention times. Hydrogen's presence alters the ionization process and spectrum for some analytes compared to helium. While this changes relative ion intensities and requires SRM transitions' re-optimization, it isn't critical as pesticide residue analysis depends on specific transition ion ratios, not spectral library fidelity. Thermo Scientific™ AutoSRM software aids in the optimization process.Figure 2 shows a full mass spectrum for deltamethrin using both helium and hydrogen. Not only is the base peak different, but the relative intensities of the remaining major ions have also changed.

Figure 2. Spectral differences for deltamethrin with helium and hydrogen as carrier gas.



Sensitivity and linearity

The sensitivity of the final method was sufficient to analyze the pesticides at 0.005 mg/kg. Figure 3 shows examples of the chromatographic peak obtained at this concentration level. Linearity was checked in the concentration range 0.005–0.500 mg/kg. According to the DG SANTE guidance document[2], the linearity evaluation is based on the back calculated concentrations. To include a point into the calibration curve, its back-calculated concentration should not deviate from the true concentration by more than 20%. More than 97% of the 181 evaluated compounds showed a linear response within the investigated concentration range in both the tested matrices. Figure 4 highlights the linearity observed for chlorfenson in baby food and for triadimenol in honey, as a representative example.

Figure 3. Sensitivity and peak shape obtained at 0.005 mg•kg^{-1} in baby food and honey for (a) pentachlorbenzene, (b) fenthion and (c) tebufenpyrad

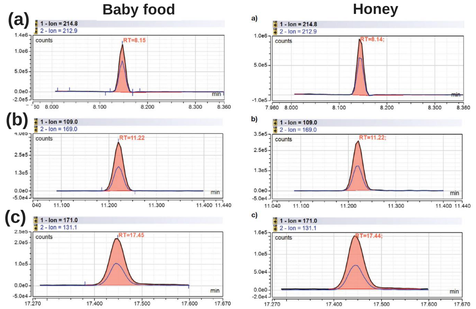
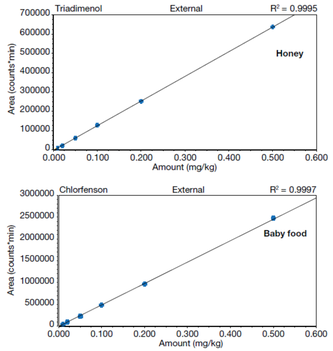


Figure 4. Matrix matched calibration curve 0.005 – 0.500 mg•kg^{-1} of tridemenol in honey and chlorfenson in baby food using hydrogen as a carrier gas



Reproducibility

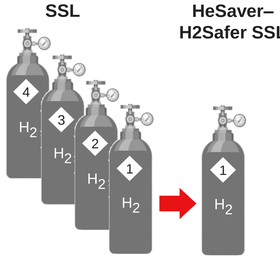
Repeatability was evaluated at two concentration levels: 0.01 mg/kg and 0.05 mg/kg. The samples were injected in quintuplicate and subsequently the relative standard deviation was calculated. In both matrices, 98% of the evaluated pesticides showed an RSD equal or lower than 10%. At 0.05 mg/kg the results were slightly better, with 99% of the analytes showing RSD ≤10%. Although the SANTE guideline allows for RSD values of up to 20% for a given compound, it is important to consider that this number applies to the full workflow, including extraction and clean-up of the sample. Therefore, an analytical method must allow for some margin to always ensure compliance. Figure 5 shows the distribution of the RSD results.

Figure 5. Repeatability (n = 5) of 181 pesticides in two matrices (baby food and honey) with hydrogen as carrier gas, compared with repeatability of pesticides in baby food using carrier helium.

Safety together with Gas Savings

Flow of H_2 carrier gas within the HeSaver-H2 Safer SSL is limited with no risk of hazardous concentrations being reached. In addition, users benefit from gas savings where consumption of carrier gas is reduced by a factor of 4 compared to the standard SSL for pesticide analysis, respectively. Figure 6 shows a visual representation of the helium savings provided.

Figure 6. Reduction in helium consumption 4-fold when using the HeSaver-H2Safer over standard SSL



Scan the QR code to use the helium saver calculator for your method

Conclusions

- The use of the HeSaver-H2Safer technology allows for a safe and compliant use of hydrogen as an alternative carrier gas in GC/GC-MS applications without the need to install a hydrogen sensor and removes any risk of unwanted reactions with the sample in the hot SSL injector.
- When used with hydrogen, the limited carrier gas consumption offered by the HeSaver-H2Safer mode permits a very controlled system demand for hydrogen, making this solution ideal for laboratories working with hydrogen generators.
- Migrating the GC-MS/MS method from helium to hydrogen requires an adaptation of method parameters to address (i.e., retention time shift and different fragmentation patterns).
- 97% of pesticides in baby food and 98% in honey showed a linear response in the concentration range 0.005–0.500 mg/kg and the relative standard deviation for N=5 repeats of each sample type at 0.01 mg/kg was equal or lower than 10% for 98% of the evaluated compounds.

References

1. Rajski Ł., Kutscher D, Ladak A. Thermo Fisher Scientific application note 002225: *Reducing running costs for GC-MS/MS analysis of pesticide residues using hydrogen carrier gas.* ***2023***.
2. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis In Food And Feed. SANTE 11312/**2021**.

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